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13. ABSTRACT (Maximum 200 Words)

In the current research plan, we proposed to study the mechanism for activation of matriptase, a membrane-bound serine protease. In non-transformed mammary epithelial cells, matriptase activation can be induced by sphingosine 1-phosphate, a blood-borne bioactive phospholipid. In addition to S1P, as an exogenous inducer, both catalytic and non-catalytic domains of matriptase could also participate in the activation of the protease. In the past one-year, we have systemically constructed matriptase mutants, and used these mutants to study the structural requirements for matriptase activation. Our data reveal that matriptase activation requires its own catalytic activity, proteolytic processing at Gly-149 in the SEA domain of the protease, glycosylation of the first CUB domain and the serine protease domain, and intact low-density lipoprotein (LDL) receptor. Its cognate inhibitor, hepatocyte growth factor activator inhibitor-1 (HAI-1), may also participate in the activation of matriptase. These results suggest that besides matriptase catalytic activity, matriptase activation requires posttranslational modification of the protease, intact non-catalytic domain, and its cognate inhibitor.

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Introduction:

Invasion of cells from one tissue to a neighboring tissue has been observed both in physiological processes, such as organ development and wound healing, and pathological processes, such as breast cancer metastases. Physiological invasion is thought to be tightly controlled. The regulatory mechanism of physiological invasion may be lost in pathologic states, particularly in malignant progression, resulting in uncontrolled invasion. Degradation of extracellular matrix (ECM) and cell migration are two key events for cellular invasion. Proteases and protease inhibitors are implicated in cellular invasion due to their potential roles in localized degradation of ECM and in the activation of latent growth/motility/angiogenesis factors, such as hepatocyte growth factor (HGF)/scatter factor (SF). HGF/SF is a major regulatory molecule for epithelial cell migration. HGF/SF and most ECM-degrading protease systems, such as the uPA system and the matrix metalloproteases are, however, expressed *in vivo* by the stromal elements of human breast cancer. Therefore, breast cancer cell invasion has been proposed to be a collaboration between epithelial cancer cells and stromal cells. In order to understand the role of epithelial cells in cellular invasion, we have characterized an epithelial-derived, breast cancer-associated, integral membrane, trypsin-like serine protease, termed matriptase (1). Matriptase has been demonstrated to activate uPA and HGF/SF (2). These observations suggest that matriptase could act as a cell surface activator to recruit and activate stromal-derived ECM-degrading proteases and motility factors. Furthermore, activation of matriptase in nontumorogenic human mammary epithelial cells (HMEC) seems to be tightly regulated by bioactive lipids, mainly sphingosine 1-phosphate (S1P) (3,4). Surprisingly, this S1P-based, physiological regulatory mechanism may be lost during breast cancer progression (5). In the current research plan, we proposed to study the role of S1P receptors in matriptase activation and the structural requirements for the activation of matriptase.

Body:

During the June 02-May 03 period, we had addressed a portion of Aim 1 and whole Aim 3 of the original proposal. Because we completely finished the Aim 3 and because a manuscript based on these work has been accepted for publication by J. Biological Chemistry, we report here only with those work in Aim 3. The work on the Aim 1 will be reported when they are more completed.

Aim 3: to investigate the role of the non-catalytic domains of matriptase in the activation of matriptase.

Year 1: We will finish all of the work proposed in Aim 3, including the determination of whether the CUB domains play the role of a "lock" for the autoactivation of matriptase, and whether matriptase may bind to itself.

Matriptase contains an N-terminal transmembrane domain, followed by a SEA (Sperm protein, Enterokinase and Agrin) domain, two tandem CUB (C1r/s, Uegf, and Bone morphogenic protein-1) domains, four tandem LDL receptor class A domains, and a catalytic serine protease domain. It also contains four putative asparagine-linked glycosylation sites, and a potential integrin-binding RGD motif is found in the first CUB domain. The protease also contains a consensus cleavage site in the SEA domain (GSVI) that is the target for a proteolytic processing cleavage in matriptase and in the mouse orthologue, epithin. This cleavage converts matriptase to smaller forms that lack the amino-terminal 149 amino acids containing the transmembrane domain, yet these processed forms are still tightly associated with the membrane. In order to investigate the roles of these domains and motifs in the activation of matriptase, we systematically constructed various mutants of human matriptase (hereafter refereed to as simply matriptase) by site-directed or deletion mutagenesis (Fig. 1). We also constructed point and deletion mutants of the human HAI-1 (hereafter refereed to as simply HAI-1) in the LDL-receptor class A domain, to test the role of this domain within the inhibitor in matriptase activation (Fig. 1).

Matriptase activation requires the catalytic activity of the serine protease domain

BT549 human breast cancer cells do not endogenously express either matriptase or HAI-1, as assessed by anti-matriptase or anti-HAI-1 western blotting (Fig. 2A and C). Therefore, the detection of transfected matriptase and HAI-1 could not be confused with endogenous proteins. In cells transfected with both wild-type matriptase and HAI-1, both the uncomplexed, processed form of matriptase (70kDa form), and the processed form, complexed to HAI-1 (120kDa complex), were observed, in addition to the full-length matriptase that

migrates at approximately 95kDa (Fig 2A). This result indicates that matriptase had been activated when co-transfected with HAI-1, since only the activated form of the enzyme binds to the inhibitor HAI-1 to form the 120 kDa complex. In addition, we confirmed this observation by western blotting with the M69 mAb, that recognizes only the two-chain, activated form of matriptase, and not the one-chain, latent form of the protease (Fig 2B). It should be pointed out that both the complexed and uncomplexed forms of matriptase were observed in M69 western blots, and the ratio of these forms varies from experiment to experiment for unknown reasons. When inactive, catalytic triad mutants of matriptase were expressed, alone or with HAI-1, the activated form of the protease was not observed, as indicated by a lack of the formation of a 120 kDa matriptase/HAI-1 complex and lack of immunoreactivity with the M69 mAb (Fig 2A and B). This data indicates that matriptase activation in BT549 cells occurs by a transactivation mechanism, and is not likely due to the activity of other proteases.

Glycosylation of the first CUB and serine protease domains is required for activation

In addition to mutations in the catalytic triad, other matriptase mutants were created to test the importance of non-catalytic domains and motifs in matriptase activation. Conservative point mutations were made in the putative glycosylation sites of matriptase (N109Q, N302Q, N485Q, and N772Q matriptase) to test their role in matriptase activation. The N772Q matriptase mutant profoundly inhibited the formation of the matriptase/HAI-1 complex (Fig. 3A) and reduced the M69 signal on western blot (Fig. 3B). The N302Q matriptase mutant also dramatically reduced the formation of the matriptase/HAI-1 complex and M69 mAb immunoreactivity, although not quite as strongly as did the N772Q matriptase mutant (Fig. 3A and B). These results strongly suggest that matriptase activation requires glycosylation of the serine protease domain and the first CUB domain. In contrast, the N109Q and N485Q matriptase glycosylation mutants did not reduce the matriptase/HAI-1 complex formation nor M69 mAb immunoreactivity (Fig. 3A and B). Overall, these results strongly suggest that matriptase glycosylation can differentially influence the level of protease activation, with glycosylation of the serine protease domain and first CUB domain potentially being important for the activation process.

Proteolytic processing in the amino-terminal SEA domain of the protease is a prerequisite for matriptase activation

Other point mutations made in matriptase included a putative proteolytic processing site within the SEA domain (G149N matriptase), important for the conversion of matriptase from a full-length 95 kDa protease to smaller molecular mass species that migrates at approximately 70 kDa, and an RGD motif (D251E matriptase) found in the first CUB domain (Fig. 1). In addition, each CUB domain was deleted individually (matriptase ΔCUB1 and matriptase ΔCUB2), and together (matriptase ΔCUB1&2), to test the function of these domains in matriptase activation.

Mutation of the putative proteolytic processing site in the SEA domain at Gly-149 (G149N matriptase) resulted in the appearance of only non-processed, full-length matriptase, as evidenced by the shift in band migration from the processed 70kDa form to the approximately 95kDa non-processed form (Fig. 3A). This is consistent with the inhibition of processing of matriptase when this site was mutated in A549 human lung carcinoma cells [1], and in the mouse orthologue of matriptase, epithin, when the homologous glycine at position 149 was mutated (6). When the amino terminal proteolytic processing of matriptase was inhibited, none of the full-length form was activated as evidenced by the lack of matriptase/HAI-1 complex formation (Fig. 3A) and a lack of M69 signal at 95kDa on western blot (Fig. 3B). This indicates that the amino-terminal proteolytic processing is a prerequisite for enzyme activation. A low level of M69 signal was observed at 70 kDa and at 120 kDa for G149N matriptase, however, indicating that a small amount of this mutant was cleaved and subsequently activated. This result confirms that the amino-terminal processed form was capable of activation, as expected. Mutation of the RGD sequence in matriptase did not affect matriptase/HAI-1 complex formation (Fig. 3A) nor the level of M69 signal on western blot (Fig. 3B), indicating that this motif is not required for matriptase activation.

To test the importance of the CUB domains in matriptase activation, the matriptase mutants lacking the first CUB domain (matriptase ΔCUB1), the second CUB domain (matriptase ΔCUB2), or both CUB domains simultaneously (matriptase ΔCUB1&2) were transfected into BT549 cells, together with HAI-1. Western blotting showed that deletion of either CUB domain or both domains together inhibited the N-terminal processing of the protease, as indicated by the appearance of a predominant higher molecular weight form, corresponding to that of full-length matriptase with CUB domain deletions (Fig. 4A). Like the G149N matriptase mutant that prevented proteolytic processing of matriptase at the N-terminus, the matriptase mutants containing single CUB deletions did not efficiently activate (Fig. 4A and B). A low level of matriptase activation was observed for matriptaseΔCUB1, consistent with some of this mutant undergoing amino-terminal processing and subsequent activation. Deletion of both CUB domains together resulted in more substantial matriptase activation, nearly equivalent to that of the wild-type matriptase (Fig 4A and B). For this mutant, more of the amino-terminal processed matriptase was observed than for either matriptaseΔCUB1 or matriptaseΔCUB2.

Activation requires intact LDL receptor class A domains

Four LDL receptor class A domains are found in matriptase. This domain is an approximately 40 amino acid long structure with three pairs of disulfide linkages, and is found in membrane receptors and many TTSP members. The prototype structure of the LDL receptor class A domain is found in the LDL receptor itself, which contains seven such domains. The crystal structure of the fifth LDL receptor class A domain in the LDL receptor revealed that this domain contains six amino acids that coordinate a Ca^{2+} atom in an octahedral arrangement, termed the calcium cage. Point mutation at critical residues in the calcium cage potently inhibits ligand binding to this domain. Point mutations in the LDL receptor class A domains of matriptase were created at an aspartic acid residue in the fifth LDL receptor class A domain that inhibits the binding of this domain with ligand, without affecting the overall folding of the molecule or adjacent domains. Thus, separate point mutations were created in each of the matriptase LDL receptor class A domains individually (D483Y matriptase, D519Y matriptase, D555Y matriptase, and D598Y matriptase), and in all four domains simultaneously (D→Yx4 matriptase). In addition, the four LDL receptor class A domains were deleted as a single unit (matriptase ΔLDLR). When transfected into BT549 cells with HAI-1, these mutants variably affected the activation of matriptase. Point mutation of each of the four matriptase LDL receptor class A domains (D482Y, D519Y, D555Y, and D598Y matriptase), or all four domains simultaneously (D→Yx4 matriptase), inhibited the activation of matriptase, as shown by a lack of the 120kDa matriptase/HAI-1 complex in M84 western blotting (Fig. 5A), and by a lack of M69 mAb immunoreactivity (Fig. 5B). However, deletion of all four domains together had the opposite effect, leading to efficient activation of matriptase and formation of discrete higher mass complexes (Fig 5A and B).

The LDL receptor class A domain of HAI-1 is essential for matriptase activation

Since the activation of matriptase required the LDL receptor class A domain within matriptase, and because this domain may be involved in protein:protein interactions, we asked whether a similar domain in HAI-1 was also essential for protease activation. Therefore, we constructed a point mutation at a critical Asp residue involved in calcium coordination in the calcium cage of the LDL receptor class A domain of HAI-1 (D349Y HAI-1) to investigate the function of this domain in matriptase activation (Fig. 1). We also created another mutant that contains a deletion of the entire LDL receptor class A domain (HAI-1 ΔLDLR). Loss of the LDL receptor class A domain function through point or deletion mutation dramatically inhibited the activation of co-transfected wild-type matriptase, as demonstrated by a lack of formation of the matriptase/HAI-1 complex (Fig 6A) and by the lack of M69 mAb immunoreactivity (Fig. 6B).

Figure 1. Mutations in matriptase and HAI-1 used to assess the function of individual domains and motifs in matriptase activation. Matriptase and HAI-1 proteins were mutated by site-directed or deletion mutagenesis to create mutant proteins that were used in transfection experiments. Mutations in the matriptase catalytic domain included substitution of His-656, Asp-711, and Ser-805 for Ala (H656A, D711A, and S805A matriptase). The location of the matriptase activation cleavage after Arg-614 is indicated by an arrow. Mutations in the LDL-receptor class A domains include point mutations that inactivate the Ca^{2+} binding cage of each domain by replacement of a critical Asp with Tyr, either singly (D482Y, D519Y, D555Y, and D598Y matriptase), or all four simultaneously (D → Yx4 matriptase). The LDL-receptor class A domains were also deleted together as a whole (matriptase ΔLDLR, deleting residues 453-603), not shown. The CUB domains were deleted individually (matripase ΔCUB1, deleting residues 213-338, and matripase ΔCUB2, deleting residues 343-444) or together (matriptase ΔCUB1&2, deleting residues 213-444), not shown. Putative N-linked glycosylation sites were mutated by replacement of Asn residues with Gln (N109Q, N302Q, N485Q, and N772Q matriptase). A putative amino-terminal proteolytic processing site at Gly-149 (G149N matriptase) was replaced with Asn, and a potential integrin-binding RGD motif was altered by replacement of Asp with Glu at position 251 (D251E matriptase). A point mutation of the LDL-receptor class A domain in the putative Ca^{2+} binding cage (D349Y HAI-1) was created by replacement of Asp-349 with Tyr, and the entire domain was also deleted (HAI-1 ΔLDLR, deleting residues 325-355), not shown. CAT, serine protease catalytic domain; LDLR, low-density lipoprotein receptor class A domain; CUB, CUB domain; SEA, SEA domain; TM, transmembrane domain; Kunitz I, Kunitz-type inhibitory domain I; Kunitz II, Kunitz-type inhibitory domain II.

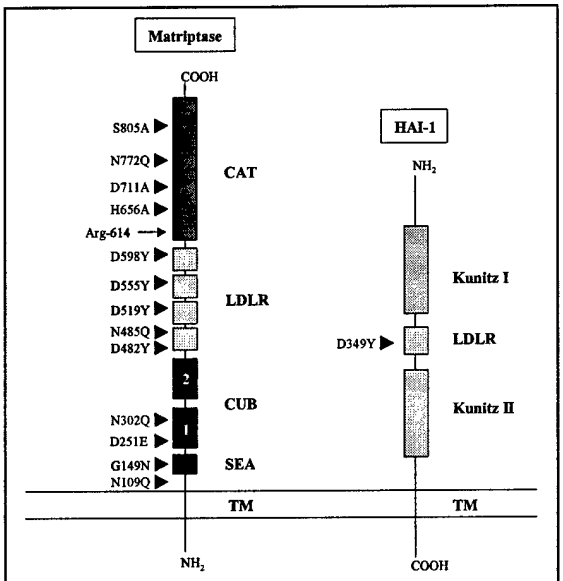

Figure 1:

Figure 2. Matriptase activation requires an intact serine protease catalytic domain. BT549 breast cancer cells were transfected with control pcDNA3.1 vector (Control), both matriptase and HAI-1 cDNAs simultaneously (Mat/HAI-1), or with catalytic triad mutants (S805A Matriptase, H656A Matriptase, and D711A Matriptase) alone or with HAI-1 as indicated. Total matriptase was detected by western blotting with the M32 mAb that recognizes total

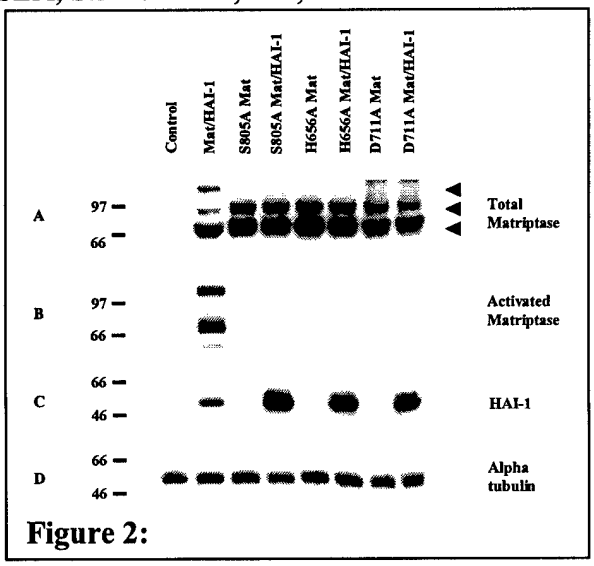

Figure 2:

(latent plus activated) matriptase (A). Activated matriptase was detected by using the M69 mAb that recognizes only activated protease (B). Activated protease could also be detected by formation of the 120 kDa matriptase/HAI-1 complex seen in panel A. HAI-1 transfection was demonstrated by western blotting using the M19 mAb (C), and equal protein loading was confirmed by using an alpha tubulin-specific mAb (D). Data are representative of three independent experiments. Arrowheads indicate the position of the 120 kDa matriptase/HAI-1 complex (top arrowhead), the 95 kDa full-length matriptase (middle arrowhead), and the 70kDa amino-terminal processed form of matriptase (bottom arrowhead).

Figure 3. Matriptase activation requires N-linked glycosylation of the first CUB and serine protease domain, and proteolytic processing of the amino terminus. BT549 breast cancer cells were transfected with control pcDNA3.1 vector (Control), both matriptase and HAI-1 cDNAs simultaneously (Mat/HAI-1), or with matriptase containing mutations in potential N-linked glycosylation sites (N109Q Mat, N302Q Mat, N485Q Mat, and N772Q Mat), in a putative N-terminal proteolytic processing site at Gly-149 (G149N Mat), or in the RGD motif within the first CUB domain (D251E Mat) together with HAI-1 as indicated. Total matriptase was detected by western blotting with the M32 mAb that recognizes total (latent plus activated) matriptase (A). Activated matriptase was detected by using the M69 mAb (B), and activated protease could also be detected by formation of the 120 kDa matriptase/HAI-1 complex seen in panel A. HAI-1 transfection was demonstrated by western blotting using the M19 mAb (C), and equal protein loading was confirmed by using an alpha tubulin-specific mAb (D). Similar results were found in three independent experiments.

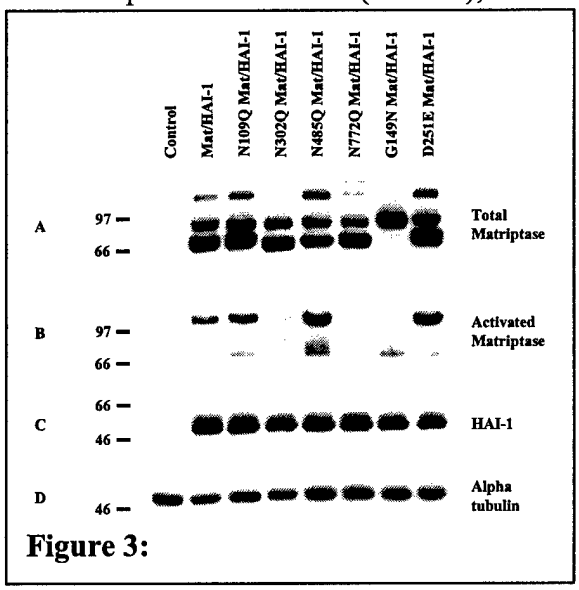

Figure 3:

Figure 4. Amino-terminal proteolytic processing of matriptase and subsequent enzyme activation requires intact CUB domains. BT549 breast cancer cells were transfected with control pcDNA3.1 vector (Control), both matriptase and HAI-1 cDNAs simultaneously (Mat/HAI-1), or with matriptase containing deletions of the first CUB domain (MatΔCUB1), the second CUB domain (MatΔCUB2), or the two CUB domains

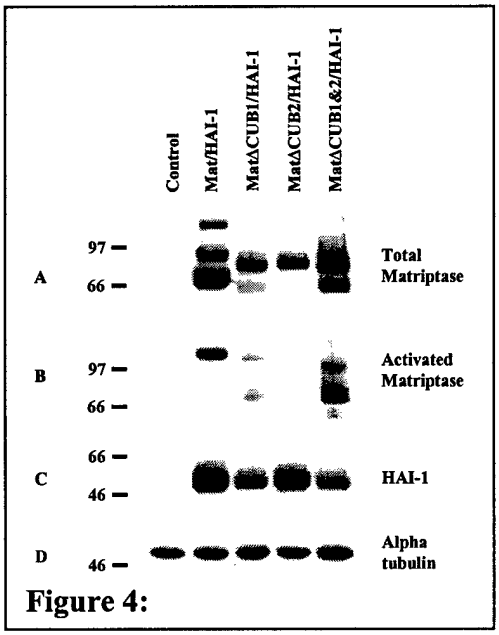

Figure 4:

simultaneously (MatΔCUB1&2), together with HAI-1 as indicated. Total matriptase was detected by western blotting with the M84 mAb (A). Activated matriptase was detected by using the M69 mAb that recognizes only activated protease (B), or by formation of the 120 kDa matriptase/HAI-1 complex seen in panel A. HAI-1 was detected by western blotting using the M19 mAb (C), and equal protein loading was confirmed by using an alpha tubulin-specific mAb (D). Data is representative of three independent experiments.

Figure 5. The LDL receptor class A domains of matriptase are required for matriptase activation. BT549 breast cancer cells were transfected with control pcDNA3.1 vector (Control), both matriptase and HAI-1 cDNAs simultaneously (Mat/HAI-1), or with matriptase containing mutations in the LDL receptor class A domain (D482YMat, D519Y Mat, D555Y Mat, D598Y Mat, D→Yx4 Mat, and MatΔLDLR) together with HAI-1 as indicated. Total matriptase was detected by western blotting with the M84 mAb that recognizes total (latent plus activated) matriptase (A). Activated matriptase was detected by using the M69 mAb (B). Activated protease could also be detected by formation of the 120 kDa matriptase/HAI-1 complex seen in panel A. HAI-1 transfection was visualized by western blotting using the M19 mAb (C). Equal protein loading was confirmed by using an alpha tubulin-specific mAb (D). Data is representative of three independent experiments.

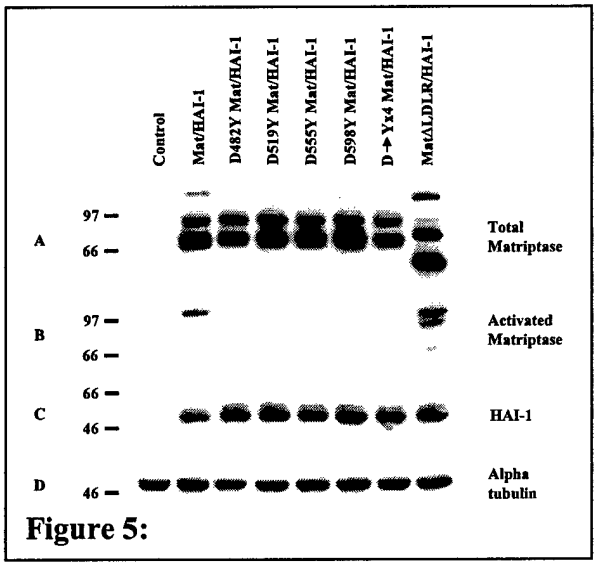

Figure 5:

Figure 6. The HAI-1 LDLR class A domain is required for matriptase activation. BT549 breast cancer cells were transfected with control pcDNA3.1 vector (Control), both matriptase and HAI-1 cDNAs simultaneously (Mat/HAI-1), or with HAI-1 LDL receptor class A domain mutants (D349Y HAI-1 and HAI-1ΔLDLR) transfected with HAI-1. Total matriptase was detected by western blotting with the M32 mAb that recognizes total (latent plus activated) matriptase (A). Activated matriptase was detected by using the M69 mAb that recognizes only activated protease (B). Activated protease could also be detected as a 120 kDa matriptase/HAI-1 complex seen in panel A. HAI-1 transfection was demonstrated by western blotting using the M19 mAb (C), and equal protein loading was confirmed by using an alpha tubulin-specific mAb (D). Data are representative of three independent experiments.

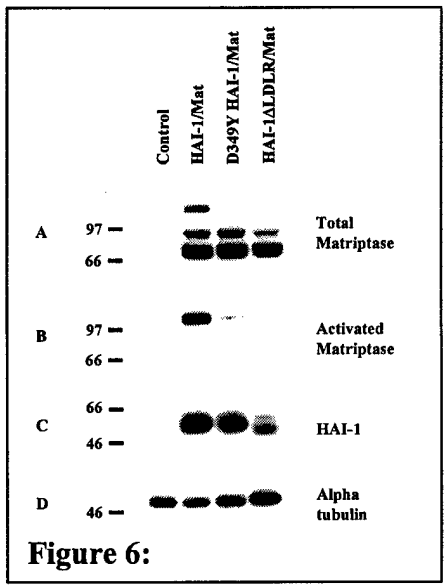

Figure 6:

Key research accomplishments:

- We have systemically constructed matriptase and HAI-1mutants.
- These mutants have been used to investigate the structural requirements for matriptase activation.
- Matriptase activation requires the catalytic activity of the serine protease domain.
- Glycosylation of the first CUB and serine protease domains is required for activation of matriptase.
- Proteolytic processing in the amino-terminal SEA domain of the protease is a prerequisite for matriptase activation.
- Activation requires intact LDL receptor class A domains.
- The LDL receptor class A domain of HAI-1 is essential for matriptase activation

Reportable outcomes:

1. Williams, C., Benaud, C., Oberst, M.D., Dickson, R.B., and Lin, C.-Y., (2002) Deregulation of matriptase activity in breast cancer cells. Proteases, Extracellular Matrix, and Cancer. AACR-Special Conference in Cancer Research, Hilton Head Island, South Carolina.
2. Benaud, C., Oberst, M., Dickson, R.B., and Lin, C.-Y., (2002) Deregulated activation of matriptase in breast cancer cells. *Clinical & Expweimwntal Metastasis*, 19:639-649.
3. Kang, J.Y., Dolled-Filhart, M., Ocal, T., Singh, B., Lin, C.-Y., Dickson, R.B., Rimm, D.L., and Camp, R.L., (2003) Tissue microarray analysis of HGF/Met pathway components reveals a role for met, matriptase, and HAI-1 in the progression of node-negative breast cancer. *Clinical Cancer Research* 63:1101-1105.
4. Oberst, M.D., Singh, B., Ozdemirli, M., Dickson, R.B., Johnson, M.D., and Lin, C.-Y. (2003) Characterization of Matriptase Expression in Normal Human Tissues. J. Histochem Cytochem (In Press).
5. Oberst, M.D., Williams, C., Johnson, M.D., Dickson, R.B., and Lin, C.-Y. (2003) The activation of matriptase requires its noncatalytic domains, serine protease domain, and its cognate inhibitor. J. Biol. Chem. (In Press).

Conclusion:

The activation of matriptase requires proteolytic cleavage at a canonical activation motif that converts the enzyme from a one-chain zymogen to an active, two-chain protease. This activating cleavage occurs via a transactivation mechanism where interaction between matriptase zymogen molecules leads to activation of the protease. Furthermore, activation of matriptase requires proteolytic processing at Gly-149 in the SEA domain of the protease, glycosylation of the first CUB domain and the serine protease domain, and intact low-density lipoprotein (LDL) receptor class A domains. Its cognate inhibitor, hepatocyte growth factor activator inhibitor-1 (HAI-1), may also participate in the activation of matriptase. These results suggest that besides matriptase catalytic activity, matriptase activation requires post-translational modification of the protease, intact noncatalytic domains, and its cognate inhibitor.

Reference:

1. Lin, C.-Y., Anders, J., Johson, M., Sang, Q. A., and Dickson, R.B. (1999) Molecular cloning of cDNA for matriptase, a matrix-degrading serine protease with trypsin-like activity. J. Biol. Chem. 274: 18231-18236.
2. Lee, S.-L., Dickson, R.B., and Lin, C.-Y., (2000) Activation of urokinase and hepatocyte growth factor by matriptase, an epithelial membrane-bound serine protease. J. Biol. Chem. 275: 36720-36725.
3. Benaund C., Dickson R.B., and Lin, C.-Y., (2001) Regulation of the activity of matriptase on epithelial cell surfaces by a blood-derived factor. Eur. J. Biochem. 268: 1439-1447.
4. Benaund C., Oberst, M., Hobson, J.P., Spiegel, S., Dickson R.B., and Lin, C.-Y., (2002) Sphingosine-1-phosphate, present in serum lipoproteins, activates matriptase. J. Biol. Chem. 277:10539-10546.
5. Benaud, C., Oberst, M., Dickson, R.B., and Lin, C.-Y., (2002) Deregulated activation of matriptase in breast cancer cells. *Clinical & Expweimwntal Metastasis*, 19:639-649.

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The activation of matriptase requires its noncatalytic domains, serine protease domain, and its cognate inhibitor.

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The activation of matriptase requires proteolytic cleavage at a canonical activation motif that converts the enzyme from a one-chain zymogen to an active, two-chain protease. In this study, matriptase, bearing a mutation in its catalytic triad was unable to undergo this activational cleavage, suggesting that the activating cleavage occurs via a transactivation mechanism where interaction between matriptase zymogen molecules leads to activation of the protease. Using additional point and deletion mutants, we showed that activation of matriptase requires proteolytic processing at Gly-149 in the SEA domain of the protease, glycosylation of the first CUB domain and the serine protease domain, and intact low-density lipoprotein (LDL) receptor class A domains. Its cognate inhibitor, hepatocyte growth factor activator inhibitor-1 (HAI-1), may also participate in the activation of matriptase, based on the observation that matriptase activation did not occur when the protease was co-expressed with HAI-1 mutated in its LDL receptor class A domain. These results suggest that besides matriptase catalytic activity, matriptase activation requires post-translational modification of the protease, intact noncatalytic domains, and its cognate inhibitor.

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